Growth and N Allocation in Rice Plants under CO$_2$ Enrichment[1]

Amane Makino*, Masayo Harada, Tetsuya Sato, Hiromi Nakano, and Tadahiko Mae

Department of Applied Biological Chemistry, Faculty of Agriculture, Tohoku University, Tsutsumidori-Amamiyamachi, Sendai 981, Japan

The effects of CO$_2$ enrichment on growth and N allocation of rice (*Oryza sativa* L.) were examined. The plants were grown hydroponically in growth chambers with a 14-h photoperiod (1000 μmol quanta m^{-2} s^{-1}) and a day/night temperature of 25/20°C. From the 28th to 70th d after germination, the plants were exposed to two CO$_2$ partial pressures, namely 36 and 100 Pa. The CO$_2$ enrichment increased the final biomass, but this was caused by a stimulation of the growth rate during the first week of the exposure to elevated CO$_2$ partial pressures. The disappearance of the initial stimulation of the growth rate was associated with a decreased leaf area ratio. Furthermore, CO$_2$ enrichment decreased the investment of N in the leaf blades, whereas the N allocation into the leaf sheaths and roots increased. Thus, the decrease in leaf N content by CO$_2$ enrichment was not due to dilution of N caused by a relative increase in the plant biomass but was due to the change in N allocation at the whole-plant level. We conclude that the growth responses of rice to CO$_2$ enrichment are mainly controlled by leaf area expansion and N allocation into leaf blades at the whole-plant level.

Whereas short-term (seconds to hours) CO$_2$ enrichment enhances the photosynthetic rate, long-term (weeks to months) CO$_2$ enrichment frequently suppresses photosynthesis (for reviews, see Stitt 1991; Bowes, 1993; Petterson and McDonald, 1994). Similarly, although plant mass is enhanced under CO$_2$ enrichment, the increase in the final plant mass by the prolonged exposure to elevated partial pressures of CO$_2$ is much less than that expected from the initial increase in photosynthesis (Badger, 1992; Koike, 1993; Masle et al., 1993; Mitchell et al., 1993). Thus, the suppression of photosynthesis by long-term CO$_2$ enrichment may be closely related to the whole-plant growth under CO$_2$ enrichment. However, the physiological and biochemical mechanisms that suppress photosynthesis in plants growing in elevated CO$_2$ partial pressures have not been elucidated.

The suppression of photosynthesis by CO$_2$ enrichment is always associated with a decrease in total leaf N content (Conroy and Hocking, 1993; Tissue et al., 1993; Delgado et al., 1994; Rogers et al., 1996; Roumet et al., 1996). In our

companion paper (Nakano et al., 1997) we report that the suppression of photosynthesis in rice (*Oryza sativa* L.) leaves grown under conditions of CO$_2$ enrichment can be simply accounted for by a decrease in leaf N. In this study we first analyzed the growth rate of rice plants under CO$_2$ enrichment in detail, and then, to elucidate the physiological implications of the decrease in leaf N content by CO$_2$ enrichment, we investigated the effects on biomass and N allocation at the whole-plant level.

MATERIALS AND METHODS

Rice (*Oryza sativa* L. cv Notohikari) plants were grown hydroponically in an environmentally controlled growth chamber (Makino et al., 1994) equipped with a CO$_2$ partial pressure controller. The chamber was maintained with a 14-h photoperiod, 25/20°C day/night temperature, 60% RH, and a PPFD of 1000 μmol quanta m^{-2} s^{-1} at plant level during the daytime. Irradiance was provided by a combination of metal halide lamps (Toshiba, Yoko DF, Tokyo) and high-output fluorescent lamps (FPR 96 EX-N/A, Panasonic, Tokyo). The rice seeds were soaked in tap water at 30°C for 2 d, and the seedlings were grown for 19 d on a plastic net floating on tap water adjusted to pH 5.5.

For experiment 1, four seedlings were transplanted to each of 24 3.5-L plastic pots containing nutrient solution. The basal nutrient solution was as previously described by Makino et al. (1988). The solution was continuously aerated, renewed once a week, and enhanced from one-third- to full-strength, depending on the plant growth (days after germination): one-third-strength, 21 to 35; one-half-strength, 35 to 42; two-thirds-strength, 42 to 49; and full-strength, 49 to 70. From the 28th day after germination, plants were grown under two CO$_2$ partial pressures, i.e. 36 $\pm$ 4 and 100 $\pm$ 5 Pa. Four plants in each pot were harvested every week between d 21 and 70, and the depletion of the nutrient solution was measured. The amount of the uptake by the plants was estimated by subtracting loss of nutrient solution without plants under the same conditions.

For experiment 2, eight seedlings were transplanted to each of 12 3.5-L plastic pots. The nutrient solution was supplied as described for experiment 1. From the 49th d after germination, two CO$_2$ treatments were imposed, 36 $\pm$

* Corresponding author; e-mail makino@biochem.tohoku.ac.jp; fax 81–22–717–8765.

Abbreviations: LAR, leaf area ratio; NAR, net assimilation rate; RGR, relative growth rate.

4 and 100 ± 5 Pa. At the same time, plants were supplied with three N concentrations (mM): 0.5 (0.25 mM NH_4NO_3), 2.0 (1.0 mM NH_4NO_3), and 8.0 (2.5 mM NH_4NO_3 plus 3.0 mM $NaNO_3$) for each CO_2 treatment. These nutrient solutions were also renewed once a week and continuously aerated. All plants were harvested on d 70.

The harvested plants were divided into main shoots and tillers and then further separated into leaf blades, leaf sheaths, and roots. Stems did not develop in any plants during the experimental period, and their dry weights were negligible. The areas of the leaf blades were measured and the tillers were counted. The blades, sheaths, and roots were oven-dried at 80°C for more than 3 d, weighed, and milled.

The RGR and the LAR were calculated from total dry weights and total leaf area of the shoots, and the NAR was calculated from the RGR and the LAR.

Dried ground materials for determination of N content were digested with H_2SO_4/H_2O_2 at 260°C. The N content was determined with Nessler's reagent and a sample of the digestion solution after the addition of potassium sodium tartrate.

RESULTS

Growth Analysis (Experiment 1)

The final dry weights of the shoots and roots of the rice plants grown in 100 Pa CO_2 were 43 and 27% greater than those of the plants grown in 36 Pa CO_2, respectively (Table I). The total leaf area and the tiller numbers tended to increase slightly in the plants grown in 100 Pa CO_2, but these differences were not statistically significant between the CO_2 treatments. Similarly, there were no differences in plant height and leaf stage between the CO_2 treatments (data not shown).

The changes in RGR, LAR, and NAR after transfer to different CO_2 partial pressures are shown in Figure 1. During the first week a marked difference in the RGR was found, but then the two CO_2 treatments showed similar RGRs. Since the LAR was not different during the 1st week, the early difference in the RGR was due to an initial stimulation of the NAR by CO_2 enrichment. From 3 weeks after the transfer to different CO_2 partial pressures, the LAR was significantly lower in the plants grown in elevated partial pressures of CO_2 than in those grown in normal partial pressures of CO_2, whereas there was no difference in the RGR. These results clearly showed that the difference in the plant shoot mass at final harvest (Table I) was caused only by an early difference in the RGR within the 1st week after CO_2 treatment.
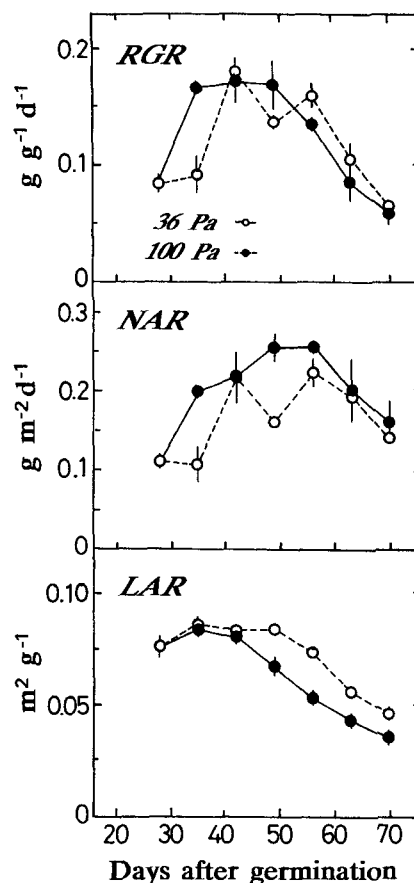

Figure 1. Changes in RGR, LAR, and NAR of rice plants for successive 7-d periods after transfer to different CO_2 partial pressures. Plants were grown hydroponically under two CO_2 partial pressures of 36 and 100 Pa. The vertical bars indicate the SE ($P < 0.05$, $n = 4$). Where no bars appear the SE falls within the size of the symbol.

Figure 2 shows the changes in total plant mass, including roots, depletion of nutrient solution, and the amount of N uptake estimated by the increase in whole-plant N. Although the depletion of nutrient solution increased with increasing plant mass, it was appreciably less in the plants grown in 100 Pa CO_2 than in the plants grown in 36 Pa CO_2. When the water-use efficiency for growth was calculated by the dry weight increase per depletion of nutrient solution per week, it was about 2-fold greater in the plants grown in elevated partial pressures of CO_2, irrespective of growth stage. The N uptake was initially stimulated by CO_2 enrichment, but this difference disappeared from 3 weeks after the transfer to elevated CO_2 levels. This disappearance was simply due to the amount of N supplied

Table I. *Plant mass, total leaf area, and tiller numbers of rice plants at final harvest (70 d after germination) in experiment 1*

Data are means ± SE ($n = 4$). Values in parentheses are the ratios relative to the data obtained with the 36-Pa CO_2 treatment.

Treatment	Shoot Mass	Root Mass	Leaf Area	Tiller No.
Pa	*g/plant*		*cm²/plant*	
36	3.46 ± 0.29[a] (100)	2.39 ± 0.34[a] (100)	251 ± 50 (100)	8.5 ± 1.1 (100)
100	4.96 ± 0.57[a] (143)	3.04 ± 0.22[a] (127)	273 ± 30 (108)	9.3 ± 0.4 (109)

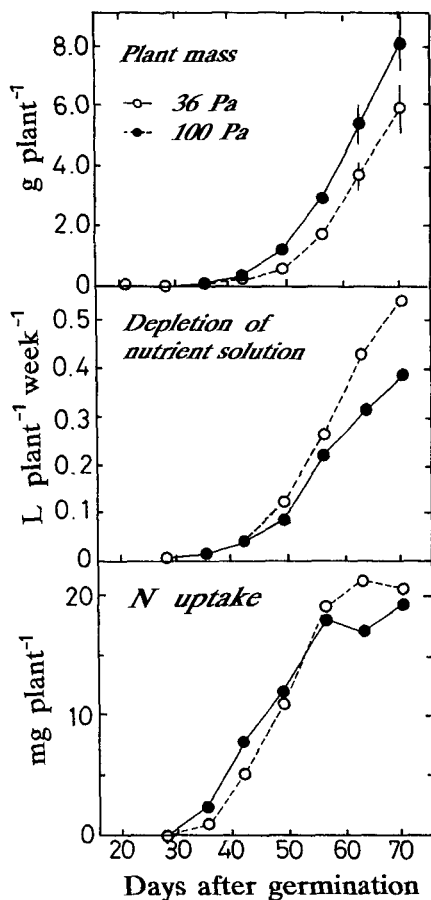[a] The difference between CO_2 treatments was significant ($P < 0.05$).

Figure 2. Changes in total dry mass of rice plants, depletion of nutrient solution per week, and the amount of N uptake calculated as the increase in total N of the whole plants including roots per week. Symbols are the same as in Figure 1. The vertical bars in the top panel indicate the SE ($P < 0.05$, $n = 4$), and no statistical analysis was done on depletion of nutrient solution and N uptake.

being completely absorbed by the plants, irrespective of the growth CO_2 levels.

Biomass Allocation and N (Experiment 2)

Table II shows the biomass allocation in the shoots for the various CO_2 and N treatments. CO_2 enrichment increased the fraction of dry matter in the leaf sheaths more

than it did in the leaf blades for all N treatments. In addition, this effect also depended on N nutrition, and it enhanced with increasing N supply. In contrast, the total leaf area at low N supply was decreased by CO_2 enrichment, but that at high N supply was little affected. CO_2 enrichment also affected the N allocation at the whole-plant level. The N investment in the leaf blades of the plants grown in elevated levels of CO_2 decreased for all N treatments, whereas CO_2 enrichment enhanced the allocation of N to the leaf sheaths and roots (Fig. 3). Since N invested into leaf blades is the most important source for photosynthesis in the whole plant, these results suggest that plants regulate photosynthesis by changing N allocation at the whole-plant level. Figure 4 shows the leaf area and N content in the leaf blades and sheaths at different leaf positions on the main culms of the plants grown at 2 mM N. CO_2 enrichment decreased the leaf area of the top, fully expanded leaf (5th d after full expansion) but had little effect on that of the second leaf (14th d) and slightly increased that of the third leaf (21st d). The N contents in the top and second leaf blades were appreciably decreased by CO_2 enrichment, but that in the third leaf blade was not significantly different. In contrast, the N contents in the leaf sheaths at all of these positions were greater in the plants grown in elevated partial pressures of CO_2. Since the top and second expanded leaves are the most photosynthetically active organs of the whole plant, the decrease in N content of these leaves may be an especially important determinant for the suppression of photosynthesis under CO_2 enrichment at the whole-plant level.

DISCUSSION

Our detailed growth analyses of rice plants showed that, although elevated CO_2 enhanced the final dry weight of the plant shoots, the RGR was similar or slightly decreased except for during the first week of growth after transfer to CO_2 enrichment (Fig. 1). Similar growth responses have often been found in several species such as *Mimulus cardinalis* (Badger, 1992), *Eucalyptus* (Wong et al., 1992), *Abutilon theophrasti* (Coleman et al., 1993), and *Nicotiana tabacum* (Masle et al., 1993). These results indicate that the increase in plant mass by CO_2 enrichment is caused only by an initial stimulation of the growth rate just after the exposure to elevated CO_2, and that the subsequent CO_2 enrichment results in the cessation of the initial stimulation of the

Table II. *Biomass of the leaf blades and the leaf sheaths and total leaf area of rice plants at final harvest (70 d after germination) in experiment 2*

Data are means ± SE ($n = 8$). Values in parentheses are the ratios relative to the data obtained with the 36-Pa CO_2 treatment.

N Treatment	Growth CO_2	Leaf Blade	Leaf Sheath	Leaf Area
mM	Pa	g/plant		cm²/plant
0.5	36	0.73 ± 0.04 (100)	1.38 ± 0.12 (100)	141 ± 8[a] (100)
	100	0.79 ± 0.09 (108)	1.55 ± 0.21 (112)	109 ± 2[a] (77)
2.0	36	0.89 ± 0.02[a] (100)	1.45 ± 0.09[a] (100)	174 ± 7[a] (100)
	100	1.08 ± 0.02[a] (121)	2.08 ± 0.15[a] (143)	148 ± 14[a] (85)
8.0	36	1.38 ± 0.17[a] (100)	1.88 ± 0.30[a] (100)	251 ± 8 (100)
	100	1.73 ± 0.13[a] (125)	2.73 ± 0.19[a] (145)	242 ± 22 (96)

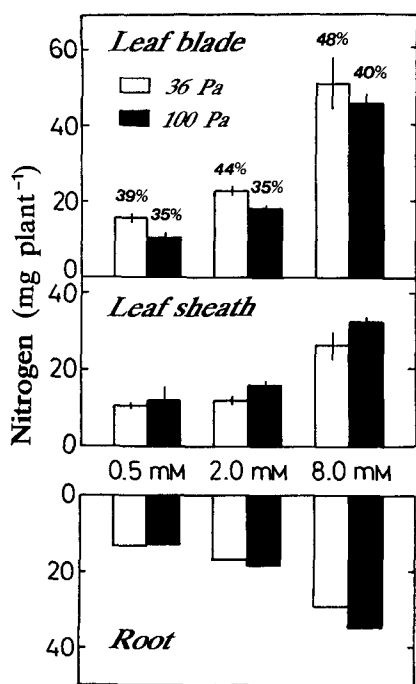[a] The difference between CO_2 treatments was significant ($P < 0.05$).

Figure 3. Total N content in leaf blades, leaf sheaths, and roots of rice plants at final harvest (70 d after germination). Plants were grown hydroponically under two CO_2 partial pressures of 36 and 100 Pa at N concentrations of 0.5, 2.0, and 8.0 mM. The percentage value above each column in the top panel shows the ratio of leaf-blade to whole-plant N. The vertical bar on each column in the top and middle panels indicates the SE ($P < 0.05$, $n = 8$). No statistical analysis was done on the data in the bottom panel.

growth rate. We propose that the decreases in the LAR and the N allocation into leaf blades at the whole-plant level observed in the present study are important factors in the suppression of the growth rate.

The disappearance of the initial increase in the RGR induced by CO_2 enrichment is frequently associated with a decrease in the LAR (Fig. 1; Wong, 1990; Badger, 1992; Wong et al., 1992; Roden and Ball, 1996). A decrease in the LAR means a relative decrease in the ratio of leaf area to plant mass, and such a response can be interpreted as one of the growth adjustments to a suppression of enhanced photosynthesis per unit leaf area. Our results also indicated that in spite of the increase in shoot mass CO_2 enrichment had little effect on leaf area (Table I) or slightly decreased it (Table II). However, many studies have shown that, although the LAR decreases, total leaf area is substantially increased by CO_2 enrichment (Wong, 1990; Wong et al., 1992; Xu et al., 1994; Roden and Ball, 1996). This phenomenon is thought to be due to enhanced branching and tillering (Mitchell et al., 1993; Koike, 1995; Samarakoon et al., 1995; Rogers et al., 1996). The leaf area in rice, in contrast to that of other plants, does not appear to be increased by elevated CO_2 partial pressures, despite the increase in tiller numbers (Imai and Murata, 1976; Morison and Gifford, 1984; Baker et al., 1990; Ziska and Teramura, 1992; Morokuma et al., 1996; Ziska et al., 1996). The reason for this difference is not known, but the effect of CO_2

enrichment on leaf area may be complicated by the fact that the water status under which plants are grown is also affected by the levels of growth CO_2. CO_2 enrichment reduces transpiration and consequently leads to improved water status for plant growth. The stimulation of leaf development by CO_2 enrichment may also be due to improved water status. For example, Samarakoon et al. (1995) reported that the enlargement of leaf area induced by elevated CO_2 levels in wheat disappeared under conditions of sufficient watering. Similar results were also obtained with pea by Paez et al. (1983). In the present study we grew the rice plants by water culture with continuous aeration. Thus, all of these results suggest the possibility that the investment in leaf area is originally reduced in the plants grown in elevated levels of CO_2 when water is abundant for growth.

In the present study with rice plants grown in elevated CO_2 partial pressures, the N investment in the leaf blades also decreased, whereas the allocation of N into the leaf sheaths and the roots increased (Fig. 3). The decrease in leaf-blade N was especially evident in upper leaves (Fig. 4). These results indicate that our rice plants reallocated N away from the leaf blades into the leaf sheaths and roots,
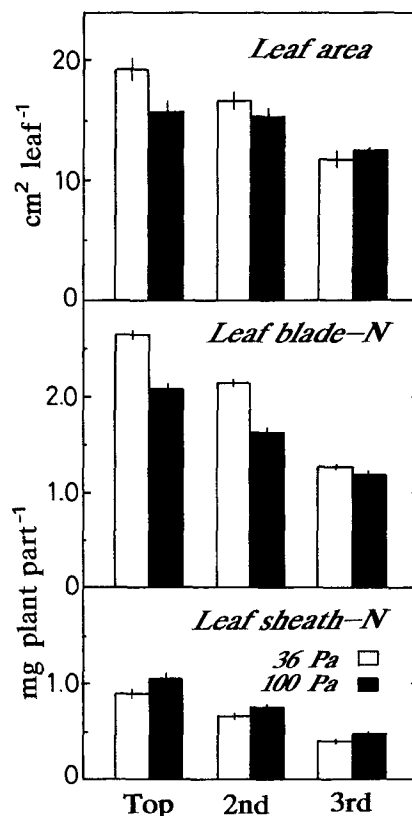

Figure 4. Leaf area and N content in the leaf blades and leaf sheaths at different leaf positions on the main culms of rice plants grown at 2 mM N (70 d after germination; final harvest). Column symbols are the same as in Figure 4. Leaves were numbered from the youngest fully expanded leaf, which was the 10th from the first leaf after germination. The vertical bar on each column indicates the SE ($P < 0.05$, $n = 4$).

and the decrease in leaf-blade N was not due to a dilution of N caused by the relative increase in total biomass, leaf area, or other growth indices. Although there was no difference in the N content of the third leaf blades (Fig. 4), this was probably due to the delay of the decrease in N content in lower leaves during senescence under CO$_2$ enrichment. The CO$_2$ enrichment decreased N allocation into upper leaves, which in turn may have reduced the N efflux from lower leaves.

A decrease in leaf N content has been commonly found in many plants grown under CO$_2$ enrichment (Conroy and Hocking, 1993; Tissue et al., 1993; Delgado et al., 1994; Rogers et al., 1996; Roumet et al., 1996). In our companion paper (Nakano et al., 1997) we report that the suppression of photosynthesis by CO$_2$ enrichment can be simply accounted for by a decrease in leaf N content. Furthermore, although an apparently selective reduction of Rubisco was found for the plants grown in high partial pressures of CO$_2$, this phenomenon was not the result of an optimization of N partitioning within a leaf under conditions of CO$_2$ enrichment but rather was the result of a decrease in leaf N content. In this study we found that the decrease in leaf N content was the result of a change in the N allocation at the morphogenic level of the whole plant. At the same time, these results suggest that plants regulate photosynthesis by changing their N allocation at the whole-plant level. Thus, such responses may be more important for plants as an adaptation strategy to high-CO$_2$ environments than biochemical responses at the level of the single leaf. We conclude that the growth responses of rice to CO$_2$ enrichment are mainly controlled by leaf area expansion and N allocation into leaf blades at the whole-plant level.

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